Form PTO-1390 (Rev. 12-29-99) US DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NO.
H 4156 PCT/US

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)
09/980192

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/04364	**May 16, 2000**	**May 25, 1999**

TITLE OF INVENTION
UTILIZATION OF MICROEMULSIONS IN FERMENTATION PROCESSES

APPLICANT(S) FOR DO/EO/US
Jean-Pierre Molitor, Matthias Wegener, Christian De Haut, Benoit Abribat, Bent Rogge

Applicant herewith submits to the United States Designated/Elected Office (EO/DO/US) the following items and other information:

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ■ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ■ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ■ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ■ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ■ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). **(UNEXECUTED)**
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ■ A FIRST preliminary amendment
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

"Express Mail Post Office to Addressee" service Mailing Label Number EL541613942US .

U.S. Application No. (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/680192	PCT/EP00/04364	H 4156 PCT/US

17. ■ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....**$1,040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....**$890.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37CFR 1.445(a)(2)) paid to USPTO **$740.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
International preliminary examination fee paid to USPTO (37CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....**$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date 37 (CFR 1.492(e)).	$ 0	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	22 - 20 =	2	2 X $18.00	$ 36	
Independent Claims	2 - 3 =	0	0 X $84.00	$ 0	
Multiple dependent claims (s)(if applicable)		0	+ $280.00	$ 0	
TOTAL OF ABOVE CALCULATIONS =				$ 926	
Reduction of ½ for filing by small entity, if applicable. A Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 0	
SUBTOTAL =				$ 926	
Processing fee of **$130.00** for furnishing the English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0	
TOTAL NATIONAL FEE =				$ 926	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 0	
TOTAL FEES ENCLOSED =				$ 926	
				Amount to be: refunded:	$----------
				charged:	$926.00

a. ☐ A check in the amount of $_____ to cover the above fees is enclosed.

b. ■ Please charge my Deposit Account No. 50-1177 in the amount of $926.00 to cover the above fees. A triplicate copy of this sheet is enclosed. Order No. 01-0741 .

c. ■ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1177 . A triplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
Cognis Corporation, Law Dept.
2500 Renaissance Blvd., Suite 200
Gulph Mills, PA 19406

SIGNATURE:

Aaron R. Ettelman
NAME ATTORNEY FOR APPLICANT

42,516
REGISTRATION NUMBER

"Express Mail " Mailing Label Number EL541613942US .

PATENT
Docket No. H 4156 PCT/US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

RE: PCT/EP00/04364
International Filing Date: May 16, 2000
Priority Date Claimed: May 25, 1999
Applicant: Molitor, et al.
Title: UTILIZATION OF MICROEMULSIONS IN FERMENTATION PROCESSES
Applicants' Reference: H 4156 PCT/US

PRELIMINARY AMENDMENT

Commissioner for Patents
Box PCT
Washington, DC 20231

ATTN: DO/EO/US

Prior to the calculation of fees and examination of the above-identified national stage application pursuant to the accompanying submission under 35 U.S.C. §371, please amend the English translation of the International Application submitted herewith, without prejudice, as follows:

In the Specification:

Please amend the instant Specification, without prejudice, as follows:

Please delete all text above line 3 of page 1, and replace the deleted matter with the following new section headings and title of the invention:

--TITLE OF THE INVENTION

Fermentation Mediums and Processes Using the Same

BACKGROUND OF THE INVENTION--

At page 3, between lines 27 and 28 thereof, please insert the following new section heading and new paragraph:

--BRIEF SUMMARY OF THE INVENTION

Preliminary Amendment of U.S. National Stage for International Application PCT/EP00/04364 filed May 16, 2000

The present invention relates, in general, to the use of microemulsions in fermentation processes.--

At page 4, between lines 6 and 7 thereof, please insert the following new section heading:

--DETAILED DESCRIPTION OF THE INVENTION--

At page 15, between lines 1 and 2, please add the following new paragraph:

--What is claimed is:--.

On a separate, new page 17, please add the following new section heading and paragraph containing an Abstract of the Disclosure:

--ABSTRACT OF THE DISCLOSURE

Reaction mediums for fermentation processes comprising: (a) a microorganism; and (b) a microemulsion, wherein the microemulsion comprises water, an emulsifier and an oil phase selected from the group consisting of (i) fatty acid alkyl esters, vegetable triglycerides, and mixtures thereof, and wherein the microemulsion has an average droplet size of from 1 to 100 nm are disclosed, as well as processes for using the same.--

In the Claims:

Please add new claims 11-32, as follows:

--11. (New) A reaction medium for fermentation processes comprising:

(a) a microorganism; and

(b) a microemulsion, wherein the microemulsion comprises water, an emulsifier and an oil phase selected from the group consisting of (i) fatty acid alkyl esters, vegetable triglycerides, and mixtures thereof, and wherein the microemulsion has an average droplet size of from 1 to 100 nm.--

--12. (New) The reaction medium according to claim 11, wherein the microemulsion has an average droplet size of from 10 to 80 nm.--

--13. (New) The reaction medium according to claim 11, wherein the microemulsion has an average droplet size of from 10 to 30 nm.--

--14. (New) The reaction medium according to claim 11, wherein the oil phase comprises a fatty acid methyl ester according to the general formula (I):

$$R^1\text{-}COO\text{-}R^2 \qquad (I)$$

wherein R^1 represents a C6-22 alkyl group and R^2 represents a methyl group.--

--15. (New) The reaction medium according to claim 11, wherein the oil phase comprises a fatty acid methyl ester selected from the group consisting of methyl oleate, methyl palmitate, methyl stearate, methyl pelargonate and mixtures thereof.--

--16. (New) The reaction medium according to claim 11, wherein the oil phase comprises an oil selected from the group consisting of coconut oil, sunflower oil, rapeseed oil and mixtures thereof.--

--17. (New) The reaction medium according to claim 11, wherein the emulsifier comprises an alkyl oligoglycoside.--

--18. (New) The reaction medium according to claim 11, wherein the emulsifier is present in an amount of from 10 to 50% by weight based on the microemulsion.--

--19. (New) The reaction medium according to claim 11, wherein the microemulsion comprises water in an amount of from 20 to 90% by weight based on the total weight of the microemulsion.--

--20. (New) The reaction medium according to claim 11, wherein the oil phase is present in an amount of from 10 to 80% by weight based on the microemulsion.--

--21. (New) A fermentation process comprising:

(a) providing a reaction medium comprising a microemulsion, wherein the microemulsion comprises water, an emulsifier and an oil phase selected from the group consisting of fatty acid alkyl esters, vegetable triglycerides, and mixtures thereof, and wherein the microemulsion has an average droplet size of from 1 to 100 nm;

(b) combining the reaction medium and a microorganism; and

(c) conducting fermentation.--

--22. (New) The fermentation process according to claim 21, wherein the reaction medium further comprises a substrate to be fermented.--

--23. (New) The fermentation process according to claim 21, wherein the oil phase is fermented by the microorganism.--

--24. (New) The fermentation process according to claim 21, wherein the microemulsion has an average droplet size of from 10 to 80 nm.--

--25. (New) The fermentation process according to claim 21, wherein the microemulsion has an average droplet size of from 10 to 30 nm.--

--26. (New) The fermentation process according to claim 21, wherein the oil phase comprises a fatty acid methyl ester according to the general formula (I):

$$R^1\text{-}COO\text{-}R^2 \qquad (I)$$

wherein R^1 represents a C6-22 alkyl group and R^2 represents a methyl group.--

--27. (New) The fermentation process according to claim 21, wherein the oil

phase comprises a fatty acid methyl ester selected from the group consisting of methyl oleate, methyl palmitate, methyl stearate, methyl pelargonate and mixtures thereof.--

--28. (New) The fermentation process according to claim 21, wherein the oil phase comprises an oil selected from the group consisting of coconut oil, sunflower oil, rapeseed oil and mixtures thereof.--

--29. (New) The fermentation process according to claim 21, wherein the emulsifier comprises an alkyl oligoglycoside.--

--30. (New) The fermentation process according to claim 21, wherein the emulsifier is present in an amount of from 10 to 50% by weight based on the microemulsion.--

--31. (New) The fermentation process according to claim 21, wherein the water is present in an amount of from 20 to 90% by weight based on the microemulsion.--

--32. (New) The fermentation process according to claim 21, wherein the oil phase is present in an amount of from 10 to 80% by weight based on the microemulsion.--

Please cancel claims 1-10, without prejudice.

REMARKS

Claims 11-32 are currently pending in the instant application.

The Specification has been amended to delete the original section headings and to insert the preferred section headings pursuant to 37 C.F.R. §1.77. A new Title of the Invention has been inserted. An Abstract of the Disclosure, in accordance with the disclosure, has been added. It is submitted that the amendments to the Specification made herein introduce no new matter. All of the amendments to the Specification constitute deletions of original section headings and/or paragraphs, and insertions or additions of new section headings and/or paragraphs. Accordingly, pursuant to 37 C.F.R. §1.121(b)(1)(iii), no separate page captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE" is necessary. A separate page containing a clean copy of the Abstract of the Disclosure has been attached for the Examiner's convenience. Entry of the amendments to the Specification made herein are therefore proper and respectfully requested.

Original claims 1-10 have been canceled and replaced with new claims 11-32 solely for the purpose of improving clarity and grammar, which may suffer in translation, and not for any reason which relates to the statutory requirements for a patent. New claims 11-32 have not been added in response to any rejection, nor in anticipation of any rejection. Applicants respectfully submit that the scope of new claims 11-32 generally corresponds to the scope of original claims 1-10, and that new claims 11-32 are no narrower than original claims 1-10. Furthermore, although a moot point in view of their cancellation, Applicants respectfully submit that original claims 1-10 satisfied the requirements of 35 U.S.C. §112, as filed. New claims 11-32 are supported by the claims as originally filed and in the Specification, for example, at page 3, line 30, through page 4, line 21; at page 5, line 13, through page 6, line 9; and in the Examples. No new matter has been introduced. All of the amendments to the Claims constitute cancellation of original claims and the addition of new claims. Accordingly, pursuant to 37 C.F.R. §1.121(c)(1)(ii), no separate page captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE" is necessary. Entry is therefore proper and respectfully requested.

Preliminary Amendment of U.S. National Stage for International Application PCT/EP00/04364 filed May 16, 2000

Prompt examination of the instant application in view of the amendments made herein is respectfully requested.

Respectfully submitted,

JEAN-PIERRE MOLITOR, *et al.*

November 15, 2001
(Date)

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ABSTRACT OF THE DISCLOSURE

Reaction mediums for fermentation processes comprising: (a) a microorganism; and (b) a microemulsion, wherein the microemulsion comprises water, an emulsifier and an oil phase selected from the group consisting of (i) fatty acid alkyl esters, vegetable triglycerides, and mixtures thereof, and wherein the microemulsion has an average droplet size of from 1 to 100 nm are disclosed, as well as processes for using the same.

Utilization of Microemulsions in Fermentation Processes

This invention relates to the use of microemulsions in fermentation processes.

Microbiological processes are being increasingly used in the synthesis of complex natural substances and other organic compounds. Such processes involve a conversion/transformation under anaerobic or aerobic conditions in which microorganisms, but especially bacteria or fungi, participate. Various terms - not always clearly distinguished from one another (such as bioconversion, biotransformation, fermentation) - are used by experts for microbiological processes. The term "fermentation" is used in the present specification for processes where microorganisms, preferably bacteria, are used for the transformation or synthesis of chemical compounds.

An important element in the development and optimization of fermentation processes is in particular the reaction medium in which the microbiological transformation takes place. The reaction medium, generally an aqueous solution or dispersion, influences above all the yield and efficiency of the process. The microorganisms need carbon, nitrogen and certain trace elements in bound form, for example calcium, iron, phosphorus or zinc, as nutrients to make successful metabolization to the required products possible. In addition, the temperature and pH regularly have to be kept in a certain, generally narrow range. Further details can be found in the manual by W. Crueger/A. Crueger, **Biotechnologie - Lehrbuch der angewandten Mikrobiologie, 2nd Edition 1984, R. Oldenbourg Verlag**. Chapter 5 of this work is particularly concerned with the fundamentals of fermentation. Accordingly, this literature reference also belongs specifically to the disclosure of the present invention. Besides high-energy sugars and derivatives thereof, natural fats and oils and

derivatives thereof, such as glycerol, glycerides, fatty acids or fatty acid esters, are additionally used as nutrients for the microorganisms in many processes. The culture media may not of course contain any ingredients which could adversely influence the metabolization of the microorganisms.

DE 37 38 812 A1, for example, describes a microbial process for the production of α,ω-dicarboxylic acids in which bacteria of the strain *Candida tropicalis* transform methyl laurate into the required dicarboxylic acids. This transformation takes place in an aqueous medium at a pH of 6.0 and at a temperature of 30°C. Besides the microorganisms, the medium contains glucose as an energy source, ethoxylated sorbitan monooleate as emulsifier, yeast extract, corn steep liquor and inorganic N and P sources. The methyl laurate is then added to the medium. There is nothing in the document in question to indicate the type of emulsion which forms in the fermenter or in which the methyl laurate is added to the fermentation broth. **EP 0 535 939 A1** describes a process for the production of ω-9-polyunsaturated fatty acids in which suitable microorganisms produce the required polyunsaturated fatty acids in an aqueous culture medium in the presence of sugars as energy sources and inorganic or organic nitrogen sources and in the presence of fatty acid methyl esters.

However, other known processes use only fatty compounds of the type described above as energy sources. This is particularly of economic interest because fatty compounds such as these are generally less expensive than sugars, starch and similar compounds. Park et al. (Park et al., **Journal of Fermentation and Bioengineering, Vol. 82, No. 2, 183-186, 1996**) describe a fermentation process for the production of tylosine in which microorganisms of the strain *Streptomyces fradiae* are used in an aqueous medium, rapeseed oil being present as sole carbon source in starting quantities of about 60 g/l.

In fermentation processes, the oxygen content in the medium or the fermentation broth also plays a key role. In aerobic processes, the oxygen

acts as a substrate. A critical factor is whether a transfer of oxygen from the gas phase to the liquid phase containing the microorganisms can take place sufficiently for the particular process. An important parameter is the specific exchange surface which, in general, is indirectly determined via the oxygen transfer coefficient k_{La} (cf. *Crueger*, Chapter 5, pages 71 et seq.). Adjustment of the optimum oxygen input is typically achieved by stirring the fermentation broth, the oxygen or the air being mixed with the liquid and the exchange of gas thus taking place at the interfaces. However, the considerable mechanical input of energy by intensive stirring, as carried out by Park et al., can also destroy parts of the culture, thus reducing the yield of the process. In addition, the dead microorganisms are themselves further degraded and can lead to poisoning of the culture through the degradation products formed so that economic production is not possible. It is known from the work of Goma and Rols (G. Goma, J.L. Rols, **Biotech. Let., Vol. 13, No. 1, pages 7 to 12, 1991**) that the use of soybean oil in fermentation processes for the production of antibiotics leads to an improvement in the oxygen transfer coefficient k_{La} which, for the same energy input (stirring), can lead to an increase in the yield of the process as a whole.

Now, the problem addressed by the present invention was to improve fermentation processes so that, on the one hand, inexpensive carbon sources could be used and, on the other hand, an adequate supply of oxygen to the microorganisms would be guaranteed without the microorganisms being exposed to unacceptably severe mechanical stressing by stirring. A way was to be found of minimizing the mechanical input of energy in fermentation processes without any reduction in yield. Preferably, the yield would be increased despite the reduced energy input.

It has now been found that the use of special fine-droplet oil-in-water (o/w) emulsions solves the problem stated above.

In a first embodiment, the present invention relates to the use of o/w

emulsions in fermentation processes, these emulsions containing at least water, emulsifiers and an oil phase, the oil phase containing one or more compounds from the groups of

a) fatty acid alkyl esters and/or

b) triglycerides of vegetable origin

and the emulsions having a droplet size of 1 to 100 nm.

The emulsions according to the invention are distinguished in particular by their droplet fineness. They are so-called microemulsions which are defined as macroscopically homogeneous, optically transparent, often low-viscosity, thermodynamically stable mixtures of at least two immiscible liquids and at least one nonionic surfactant or one ionic surfactant which preferably contains two hydrophobic residues. The formation of a microemulsion involves a situation where the oil/water interfacial tension approaches the value zero. In addition to at least one nonionic surfactant, other co-surfactants generally have to be added to achieve this special form of emulsion, cf. the literature reference **"Introduction to Colloid and Surface Chemistry", D.J. Shaw, Butterworth, 1992, pages 269 and 270**. The droplet size of the emulsions used in accordance with the invention is in the range from 1 to 100 nm, preferably in the range from 10 to 80 nm and more particularly in the range from 10 to 30 nm. The fineness of the oil droplets leads to a large surface between the oil and water phases and thus provides for rapid contact between the microorganisms present in the aqueous phase and the oil phase containing the nutrients. The large surface also simplifies the exchange of gases, particularly oxygen and CO_2. In addition, the viscosity of the emulsion and hence of the entire fermentation medium decreases. As a result, the stirring speed of the fermentation medium can be distinctly reduced so that the yield of the fermentation process can be increased.

According to the invention, the microemulsions are added to the aqueous fermentation medium containing the microorganisms and

optionally other auxiliaries and additives. The details of this process, more especially the addition rate and added quantity of the emulsion, are determined by the microorganism strains and the fermentation process selected and may be adapted by the expert to suit the particular circumstances.

Besides water, the microemulsions contain an oil phase which contains compounds from the group of fatty acid alkyl esters a) or native vegetable oils and derivatives thereof b). Groups a) and b) are hydrophobic, water-insoluble or substantially water-insoluble compounds which may serve as nutrients, i.e. energy sources, for the bacteria used in the fermentation process, but which may also be starting materials (substrates) for the products to be obtained by bioconversion.

Suitable methyl esters of group a) are derived in particular from saturated, unsaturated, linear or branched fatty acids containing a total of 7 to 23 carbon atoms. In other words, they are compounds corresponding to formula (I):

$$R^1\text{-}COO\text{-}R^2 \qquad (I)$$

where R^1 is a C_{6-22} alkyl group and R^2 is a C_{1-4} alkyl group. Methyl and ethyl groups are preferred. Methyl esters are particularly preferred as component a). The esters of formula (I) or rather the methyl esters may be obtained in known manner, for example by transesterification of triglycerides with methanol and subsequent distillation. Suitable fatty acids are caproic acid, heptanoic acid, caprylic acid, pelargonic acid, capric acid, undecanoic acid, lauric acid, tridecanoic acid, myristic acid, pentadecanoic acid, palmitic acid, heptadecanoic acid, stearic acid, nonadecanoic acid, arachic acid and behenic acid. Unsaturated representatives are, for example, lauroleic acid, myristoleic acid, palmitoleic acid, petroselaidic acid, oleic acid, elaidic acid, ricinoleic acid, linoleic acid, linolaidic acid,

linolenic acid, gadoleic acid, arachidonic acid and erucic acid. Mixtures of the methyl esters of these acids are also suitable. It is particularly preferred to use microemulsions containing methyl esters from the group consisting of methyl oleate, methyl palmitate, methyl stearate and/or methyl pelargonate. However, methyl esters based on natural fatty acid mixtures obtainable, for example, from linseed oil, coconut oil, palm oil, palm kernel oil, olive oil, castor oil, rapeseed oil, sesame oil, soybean oil or sunflower oil (in the case of rapeseed and sunflower oil, new and old plants) may also be used.

Suitable group b) compounds are native oils of vegetable origin. These are essentially triglyceride mixtures where the glycerol is always completely esterified with relatively long-chain fatty acids. Particularly suitable vegetable oils are selected from the group consisting of peanut oil, coconut oil, linseed oil, palm oil, olive oil, palm kernel oil, castor oil, rapeseed oil, sesame oil, soybean oil and sunflower oil.

Peanut oil contains on average (based on fatty acid) 54% by weight oleic acid, 24% by weight linoleic acid, 1% by weight linolenic acid, 1% by weight arachic acid, 10% by weight palmitic acid and 4% by weight stearic acid and has a melting point of 2 to 3°C. Linseed oil typically contains 5% by weight palmitic acid, 4% by weight stearic acid, 22% by weight oleic acid, 17% by weight linoleic acid and 52% by weight linolenic acid and has an iodine value of 155 to 205, a saponification value of 188 to 196 and a melting point of about -20°C. Coconut oil contains ca. 0.2 to 1% by weight hexanoic acid, 5 to 8% by weight octanoic acid, 6 to 9% by weight decanoic acid, 45 to 51% by weight lauric acid, 16 to 19% by weight myristic acid, 9 to 11% by weight palmitic acid, 2 to 3% by weight stearic acid, less than 0.5% by weight behenic acid, 8 to 10% by weight oleic acid and up to1% by weight linoleic acid as fatty acid components. It has an iodine value of 7.5 to 9.5, a saponification value of 0.88 to 0.90 and a melting point of 20 to 23°C. Olive oil predominantly contains oleic acid (cf.

Lebensmittelchem. Gerichtl. Chem., 39, 112 to 114, 1985). Palm oil contains ca. 2% by weight myristic acid, 42% by weight palmitic acid, 5% by weight stearic acid, 41% by weight oleic acid, 10% by weight linoleic acid as fatty acid components. Palm kernel oil typically has the following composition in relation to its fatty acid spectrum: 9% by weight caproic/caprylic/capric acid, 50% by weight lauric acid, 15% by weight myristic acid, 7% by weight palmitic acid, 2% by weight stearic acid, 15% by weight oleic acid and 1% by weight linoleic acid. Rapeseed oil typically contains ca. 48% by weight erucic acid, 15% by weight oleic acid, 14% by weight linoleic acid, 8% by weight linolenic acid, 5% by weight eicosenoic acid, 3% by weight palmitic acid, 2% by weight hexadecenoic acid and 1% by weight docosadienoic acid as fatty acid components. Rapeseed oil from new plants is richer in the unsaturated components. Typical fatty acid components here are erucic acid 0.5% by weight, oleic acid 63% by weight, linoleic acid 20% by weight, linolenic acid 9% by weight, eicosenoic acid 1% by weight, palmitic acid 4% by weight, hexadecenoic acid 2% by weight and docosadienoic acid 1% by weight. 80 to 85% by weight of castor oil consists of the glyceride of ricinoleic acid. Castor oil also contains ca. 7% by weight glycerides of oleic acid, 3% by weight glycerides of linoleic acid and ca. 2% by weight glycerides of palmitic and stearic acid. 55 to 65% by weight of the total fatty acids in soybean oil are polyunsaturated acids, more particularly linoleic and linolenic acid. The situation with sunflower oil is similar, its typical fatty acid spectrum - based on total fatty acid - being as follows: ca. 1% by weight myristic acid, 3 to 10% by weight palmitic acid, 14 to 65% by weight oleic acid and 20 to 75% by weight linoleic acid.

All the above numerical data on the fatty acid components of the triglycerides are dependent on the quality of the raw materials and, accordingly, can vary. Microemulsions containing group b) nutrients selected from coconut oil, sunflower oil and/or rapeseed oil are particularly preferred.

Important constituents of the microemulsions used in accordance with the invention are the emulsifiers and emulsifier systems used. Nonionic emulsifiers, more particularly ethoxylated fatty alcohols and fatty acids, are preferably used as emulsifiers.

Fatty alcohol ethoxylates in the context of the teaching according to the invention correspond to formula (II):

$$R^3\text{-}O\text{-}(CH_2CH_2O)_n\text{-}H \qquad (II)$$

in which R^3 is a linear or branched, saturated or unsaturated alkyl group containing 6 to 24 carbon atoms and n is a number of 1 to 50. Compounds of formula (II) where n is a number of 1 to 35 and more particularly a number of 1 to 15 are particularly preferred. Other particularly preferred compounds of formula (II) are those where R^3 is an alkyl group containing 16 to 22 carbon atoms.

The compounds of formula (II) are obtained in known manner by reaction of fatty alcohols under pressure with ethylene oxide, optionally in the presence of acidic or basic catalysts. Typical examples are caproic alcohol, caprylic alcohol, 2-ethyl hexyl alcohol, capric alcohol, lauryl alcohol, isotridecyl alcohol, myristyl alcohol, cetyl alcohol, palmitoleyl alcohol, stearyl alcohol, isostearyl alcohol, oleyl alcohol, elaidyl alcohol, petroselinyl alcohol, linolyl alcohol, linolenyl alcohol, elaeostearyl alcohol, arachyl alcohol, gadoleyl alcohol, behenyl alcohol, erucyl alcohol and brassidyl alcohol and the technical mixtures thereof obtained, for example, in the high-pressure hydrogenation of technical methyl esters based on fats and oils or aldehydes from Roelen's oxosynthesis and as monomer fraction in the dimerization of unsaturated fatty alcohols. Technical fatty alcohols containing 12 to 18 carbon atoms, such as for example coconut oil, palm oil, palm kernel oil or tallow fatty alcohol, are preferred.

Fatty acid ethoxylates which may also be used as emulsifier or as an

emulsifier component preferably correspond to formula (III):

$$R^4CO_2(CH_2CH_2O)_mH \qquad (III)$$

where R^4 is a linear or branched alkyl group containing 12 to 22 carbon atoms and m is a number of 5 to 50 and preferably 15 to 35. Typical examples are products of the addition of 20 to 30 moles ethylene oxide onto lauric acid, isotridecanoic acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, isostearic acid, oleic acid, elaidic acid, petroselic acid, linoleic acid, linolenic acid, elaeostearic acid, arachic acid, gadoleic acid, behenic acid and erucic acid and the technical mixtures thereof obtained for example in the pressure hydrolysis of natural fats and oils or in the reduction of aldehydes from Roelen's oxosynthesis. Products of the addition of 20 to 30 moles ethylene oxide onto C_{16-18} fatty acids are preferably used.

Partial glycerides which may also be used as emulsifiers preferably correspond to formula (IV):

$$\begin{array}{l} CH_2O(CH_2CH_2O)_x\text{-}COR^5 \\ | \\ CH\text{-}O(CH_2CH_2O)_yH \\ | \\ CH_2O(CH_2CH_2O)_z\text{-}H \end{array} \qquad (IV)$$

where CO R^5 is a linear or branched acyl group containing 12 to 22 carbon atoms and x, y and z together stand for 0 or for numbers of 1 to 50 and preferably 15 to 35. Typical examples of partial glycerides suitable for the purposes of the invention are lauric acid monoglyceride, coconut fatty acid monoglyceride, palmitic acid monoglyceride, stearic acid monoglyceride, isostearic acid monoglyceride, oleic acid monoglyceride, and tallow fatty acid monoglyceride and addition products thereof with 5 to 50 and preferably 20 to 30 moles ethylene oxide. Monoglycerides or technical mono/diglyceride mixtures predominantly containing monoglycerides (IV)

where CO R^5 is a linear acyl group containing 16 to 18 carbon atoms, are preferably used.

Other suitable emulsifiers are, for example, nonionic surfactants from at least one of the following groups:

(I) products of the addition of 2 to 30 moles of ethylene oxide and/or 0 to 5 moles of propylene oxide onto linear fatty alcohols containing 8 to 22 carbon atoms;

(II) glycerol monoesters and diesters and sorbitan monoesters and diesters of saturated and unsaturated fatty acids containing 6 to 22 carbon atoms and ethylene oxide adducts thereof;

(III) alkyl mono- and oligoglycosides containing 8 to 22 carbon atoms in the alkyl group and ethoxylated analogs thereof;

(IV) products of the addition of 15 to 60 moles of ethylene oxide onto castor oil and/or hydrogenated castor oil;

(V) polyol esters and, in particular, polyglycerol esters such as, for example, polyglycerol polyricinoleate or polyglycerol poly-12-hydroxystearate. Mixtures of compounds from several of these classes are also suitable;

(VI) products of the addition of 2 to 15 moles of ethylene oxide onto castor oil and/or hydrogenated castor oil;

(VII) partial esters based on linear, branched, unsaturated or saturated $C_{6/22}$ fatty acids, ricinoleic acid and 12-hydroxystearic acid and glycerol, polyglycerol, pentaerythritol, dipentaerythritol, sugar alcohols (for example sorbitol) and polyglucosides (for example cellulose);

(VIII) wool wax alcohols;

(IX) polyalkylene glycols.

The addition products of ethylene oxide and/or propylene oxide onto glycerol mono- and diesters and sorbitan mono- and diesters of fatty acids

or onto castor oil are known commercially available products. They are homolog mixtures of which the average degree of alkoxylation corresponds to the ratio between the quantities of ethylene oxide and/or propylene oxide and substrate with which the addition reaction is carried out.

It is particularly preferred to use emulsifiers of group (III), i.e. alkyl glycosides. Alkyl and alkenyl oligoglycosides are known nonionic surfactants which correspond to formula (V):

$$R^6O\text{-}[G]_p \qquad (V)$$

in which R^6 is an alkyl and/or alkenyl radical containing 4 to 22 carbon atoms, G is a sugar unit containing 5 or 6 carbon atoms and p is a number of 1 to 10. They may be obtained by the relevant methods of preparative organic chemistry. The synoptic articles by Biermann et al. in **Starch/Stärke 45, 281 (1993)**, B. Salka in **Cosm. Toil. 108, 89 (1993)** and J. Kähre et al. in **SÖFW-Journal, No. 8, 598 (1995)** are cited as representative of the extensive literature available on the subject.

The alkyl and/or alkenyl oligoglycosides may be derived from aldoses or ketoses containing 5 or 6 carbon atoms, preferably glucose. Accordingly, the preferred alkyl and/or alkenyl oligoglycosides are alkyl and/or alkenyl oligoglucosides. The index p in general formula (V) indicates the degree of oligomerization (DP), i.e. the distribution of mono- and oligoglycosides, and is a number of 1 to 10. Whereas p in a given compound must always be an integer and, above all, may assume a value of 1 to 6, the value p for a certain alkyl oligoglycoside is an analytically determined calculated quantity which is generally a broken number. Alkyl and/or alkenyl oligoglycosides having an average degree of oligomerization p of 1.1 to 3.0 are preferably used. Alkyl and/or alkenyl oligoglycosides having a degree of oligomerization of less than 1.7 and, more particularly, between 1.2 and 1.4 are preferred from the applicational point of view. The

alkyl or alkenyl radical R^6 may be derived from primary alcohols containing 4 to 11 and preferably 8 to 10 carbon atoms. Typical examples are butanol, caproic alcohol, caprylic alcohol, capric alcohol and undecyl alcohol and the technical mixtures thereof obtained, for example, in the hydrogenation of technical fatty acid methyl esters or in the hydrogenation of aldehydes from Roelen's oxosynthesis. Alkyl oligoglucosides having a chain length of C_8 to C_{10} (DP = 1 to 3), which are obtained as first runnings in the separation of technical C_{8-18} coconut oil fatty alcohol by distillation and which may contain less than 6% by weight of C_{12} alcohol as an impurity, and also alkyl oligoglucosides based on technical $C_{9/11}$ oxoalcohols (DP = 1 to 3) are preferred. In addition, the alkyl or alkenyl radical R^6 may also be derived from primary alcohols containing 12 to 22 and preferably 12 to 14 carbon atoms. Typical examples are lauryl alcohol, myristyl alcohol, cetyl alcohol, palmitoleyl alcohol, stearyl alcohol, isostearyl alcohol, oleyl alcohol, elaidyl alcohol, petroselinyl alcohol, arachyl alcohol, gadoleyl alcohol, behenyl alcohol, erucyl alcohol, brassidyl alcohol and technical mixtures thereof which may be obtained as described above. Alkyl oligoglucosides based on hydrogenated $C_{12/14}$ cocoalcohol with a DP of 1 to 3 are preferred. If alkyl glycosides of formula (V) are used as emulsifiers, it can be of advantage to use small quantities of polyhydroxycarboxylic acids, preferably citric acid, with them as formulation aids. In that case, the polyhydroxy acids are used in quantities of 0.1 to 3.0% by weight and preferably in quantities of 0.1 to 1.0% by weight.

The microemulsions used in accordance with the invention preferably contain 20 to 90% by weight of water, more preferably 30 to 80% by weight and most preferably 30 to 60% by weight of water. The balance to 100% by weight is made up of oil phase and emulsifiers and optionally other auxiliaries and additives. The oil phase itself is present in quantities of preferably 10 to 80% by weight, more preferably 20 to 70% by weight and most preferably 25 to 55% by weight. In a preferred embodiment, the

oil phase exclusively contains components a) or b) or mixtures of these components. The use of emulsions containing the oil and water phases in a ratio by weight of 1:1 is particularly preferred. The emulsifiers or emulsifier systems are present in quantities of preferably 10 to 50% by weight, more preferably 15 to 45% by weight and most preferably 20 to 40% by weight.

According to the invention, the described microemulsions may be used in fermentation processes of all kinds. Any of the various processes known to the expert, for example batch or fed batch and continuous fermentation, may be used. In addition, any of the fermenter systems known to the expert may be used. For details, see *Crueger*, pages 50 to 70. Moreover, the use of the microemulsions is not confined to specific microorganisms. On the contrary, the emulsions may be used for the production or transformation of any of the compounds known to the expert through fermentation. Apart from the conventional fermentation processes which are mainly used for the synthesis of antibiotics (cf. *Crueger*, pages 197 to 242), the described emulsions are also suitable for use in microbial transformations (bioconversions), for example the transformation of steroids and sterols, antibiotics and pesticides or the production of vitamins (cf. *Crueger*, pages 254 to 273). However, the described emulsions are preferably used in fermentation processes for the production of antibiotics, for example cephalosporins, tylosine or erythromycin.

In general, the microemulsions are suitably added to the aqueous fermentation broth containing the microorganisms and the nitrogen source and trace elements and optionally other auxiliaries, especially defoamers. Suitable nitrogen sources are, for example, peptone, yeast or malt extract, corn steep liquor, urea or lecithins. The trace elements may be present in the form of inorganic salts, for example sodium or potassium nitrate, ammonium nitrate, ammonium sulfate, iron sulfate, etc. It can also be of advantage to add other additives, such as defoamers or nitrogen sources,

to the microemulsions themselves.

Examples

Various microemulsions were prepared by mixing the starting materials. Their compositions are shown in Table 1 below. The droplet size was measured with a Malvern Mastersizer 2000. The emulsions are suitable, for example, as sole nutrient source for fermentation processes and may be directly added to the aqueous fermentation broth.

Table 1a

	% by weight	% by weight	% by weight	% by weight
Methyl oleate				32.44
Methyl laurate			30.95	
Methyl pelargonate		29.61		
Rapeseed oil fatty acid methyl ester	32.35			
Water	34.27	32.04	30.24	34.18
Alkyl glycoside	25.22	31.55	30.95	25.3
Glycerol oleate	7.91	6.55	7.62	7.84
Citric acid	0.25	0.24	0.24	0.24
Appearance	Clear	Clear	Clear	Clear
Droplet size	< 80 nm	< 80 nm	< 80 nm	< 80 nm

CLAIMS

1. The use of o/w emulsions containing at least water, emulsifiers and an oil phase containing one or more compounds selected from the groups of

a) fatty acid alkyl esters and/or

b) triglycerides of vegetable origin

characterized in that the emulsion has a droplet size of 1 to 100 nm, in fermentation processes.

2. The use claimed in claim 1, characterized in that fatty acid methyl esters are used as component a).

3. The use claimed in claim 1 or 2, characterized in that emulsions with a mean droplet size of 10 to 80 nm and preferably 10 to 50 nm are used.

4. The use claimed in claims 1 to 3, characterized in that emulsions containing water in quantities of 20 to 90% by weight, preferably 30 to 80% by weight and more particularly 30 to 60% by weight are used.

5. The use claimed in claims 1 to 4, characterized in that emulsions containing the oil phase in quantities of 10 to 80% by weight, preferably 20 to 70% by weight and more particularly 25 to 55% by weight are used.

6. The use claimed in claims 1 to 5, characterized in that emulsions containing fatty acid methyl esters of formula (I):

$$R^1\text{-}COO\text{-}R^2 \qquad (I)$$

in which R^1 is a $C_{6\text{-}22}$ alkyl group and R^2 is a methyl group, in the oil phase are used.

7. The use claimed in claims 1 to 6, characterized in that emulsions containing methyl oleate, methyl palmitate, methyl stearate and/or methyl pelargonate in the oil phase are used.

8. The use claimed in claims 1 to 7, characterized in that emulsions

containing coconut oil, sunflower oil and/or rapeseed oil in the oil phase are used.

9. The use claimed in claims 1 to 8, characterized in that emulsions containing alkyl oligoglycosides as emulsifiers are used.

10. The use claimed in claims 1 to 9, characterized in that emulsions containing emulsifiers in quantities of 10 to 50% by weight, preferably in quantities of 15 to 40% by weight and more particularly in quantities of 20 to 35% by weight are used.

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
Internationales Büro

(43) Internationales Veröffentlichungsdatum
30. November 2000 (30.11.2000)

PCT

(10) Internationale Veröffentlichungsnummer
WO 00/71672 A1

(51) Internationale Patentklassifikation[7]: C12N 1/00, C12P 1/00

(21) Internationales Aktenzeichen: PCT/EP00/04364

(22) Internationales Anmeldedatum:
16. Mai 2000 (16.05.2000)

(25) Einreichungssprache: Deutsch

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
199 23 784.0 25. Mai 1999 (25.05.1999) DE

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(84) Bestimmungsstaaten *(regional)*: europäisches Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(54) **Title:** UTILIZATION OF MICROEMULSIONS IN FERMENTATION PROCESSES

(54) **Bezeichnung:** VERWENDUNG VON MIKROEMULSIONEN IN FERMENTATIONSVERFAHREN

(57) **Abstract:** The invention relates to the utilization of O/W emulsions in fermentation processes, said emulsions containing at least water, emulsifying agents and an oil phase that contains one or more compounds selected from the group consisting of: a) fatty acid alkyl ester and/or b) triglycerides of vegetable origin, wherein the emulsions have an average drop size ranging from 1 to 100 nm.

(57) **Zusammenfassung:** Verwendung von O/W-Emulsionen, enthaltend mindestens Wasser, Emulgatoren sowie eine Ölphase, die eine oder mehrere Verbindungen enthält, ausgewählt aus den Gruppen a) der Fettsäurealkylester und/oder b) der Triglyceride pflanzlichen Ursprungs, wobei die Emulsionen eine mittlere Tröpfchengrösse im Bereich von 1 bis 100 nm aufweisen, in Fermentationsverfahren.

WO 00/71672 A1

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PTO/SB/01 (6-95)
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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing

Attorney Docket Number	H 4156 PCT/US
First Named Inventor	MOLITOR, Jean-Pierre
COMPLETE IF KNOWN	
Application Number	09/980,192
Filing Date	04/15/2002
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:
My residence, post office address, and citizenship are as stated below next to my name.
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

UTILIZATION OF MICROEMULSIONS IN FERMENTATION PROCESSES

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY) 05/16/2000 as United States Application Number or PCT International Application Number PCT/EP00/04364 and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY	Priority Not Claimed	Certified Copy Attached? YES	Certified Copy Attached? NO
199 23 784.0	DE	05/25/1999	☐	☐	☒

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Page 2

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U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*
	PCT/EP00/04364	05/16/2000	

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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DECLARATION

ADDITIONAL INVENTOR(S) Supplemental Sheet

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